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The phytochemical, antibacterial and antioxidant activity of five medicinal plants against the wound infecting bacteria

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Leaf extracts of Senna italica, Ricinus communis, Lantana camara, Lippia javanica and Ziziphus mucronata were screened for biological activity against bacteria which infect wounds. The leaves were extracted using different solvents of varying polarity (hexane, dichloromethane, acetone and methanol). Phytochemical analyses of the extracts were performed using thin layer chromatography (TLC). The extracts were loaded on TLC plates and developed in three solvent systems that is benzene/ethanol/ammonium solution (BEA), chloroform/ethyl acetate/formic acid (CEF) and ethyl acetate/methanol/water (EMW). Antibacterial activity of the plants was evaluated using micro-dilution and bioautography methods. The test organisms used were Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus and Enterococcus faecalis. Acetone extracts were chosen for antioxidant activity. Methanol was the best extractant, followed by acetone and dichloromethane (DCM). In the phytochemical analysis, more compounds were observed on BEA, followed by EMW and CEF plates. Lantana camara had no activity against any of the bacteria used. P. aeruginosa was the most resistant bacterium with only two plants active against it. E. faecalis and E. coli were sensitive to the extracts. More antibacterial compounds were observed on BEA plates against all the test bacteria in bioautographic method. The R_f values calculated from bioautography indicated that the selected plants have different active compounds. The most active compounds were from S. italica and Z. mucronata. BEA and EMW plates had good antioxidant activity. No antioxidant activity was observed on the CEF plate. Most extracts were active against wound pathogens; their application on the wound area may prevent infection. Further studies are required to identify the active compounds in the plant extracts which showed significant anti-bacterial activities.

Key words: Thin layer chromatography (TLC), plant extract, bacteria.

INTRODUCTION

Since the beginning of time, man has been dependant on plants for medicinal purposes. Plants have been used to treat a variety of ailments and the introduction of orthodox medicine did not affect their use (Chah et al., 2006). Currently, it is estimated that 80% of the world's population is still dependant on plants for their health needs. About 60% of the people in South Africa use plants in conjunction with pharmaceuticals (McGaw and Eloff, 2008). In many under developed and developing countries, rural people are still forced to use traditional medicine due to inaccessibility of healthcare facilities, escalating costs of modern medicine and prior knowledge

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of plants used to treat particular ailments (Muthu et al., 2006; Steenkamp et al., 2004). Furthermore, pathogens have acquired resistance towards many antibiotics. Some antibiotics, while effective, have been associated with adverse effects such as hypersensitivity, immune-suppression and allergic reactions (Ahmad et al., 1998). There is an increased demand for cheap medicines in South Africa due to high unemployment rate and increased human immune virus (HIV) infections, resulting in increase in the sale of medicinal plant material (Shai et al., 2008).

Many plants produce secondary metabolites which act against wound-infecting bacteria and parasites (McGaw and Eloff, 2008). Plants are a good source of antioxidants and also cure various disorders associated with wound inflammation (Mathur et al., 2011). Identification and studies on active compounds from plants are now of major interest to the scientific community (Shai et al., 2008). These include compounds such as diterpenoid found in *Jatropha zeyheri* which showed antibacterial activity against *Streptococcus pyogenes* and some fungi. Tannins, found in a number of plants used in wound healing were also found to have antibacterial activity (Luseba et al., 2007).

There are several of bacteria which are responsible for wound infections resulting in derailed wound healing. Some bacterial wound infections can be characterized by a change in the colour of the skin around the wound area and formation of lesions. One such infection is that of Pseudomonas aeruginosa, characterized by a green pigment which later becomes a black lesion (Al-Akayleh, 1999). Pathogenic bacterium such as Staphylococcus aureus has been isolated from diabetic wounds and foot ulcers (Hirsh et al., 2008). Enterococcus faecalis have also been isolated from surgical wounds (Giacometti et al., 2000). Todar (2007) found extraintestinal Escherichia coli in surgical wounds. This study aimed at evaluating the phytochemical, antibacterial and antioxidant activity of Lantana camara, Lippia javanica, Ricinus communis, Senna italica and Ziziphus mucronata against bacteria which infect wounds (Table 1).

MATERIALS AND METHODS

Plant collection

The leaves of *L. camara, L. javanica, R. communis, S. italica* and *Z. mucronata,* respectively were collected at the University of Limpopo and were stored at room temperature in a well ventilated room. The leaves were ground to powder using mortar and pestle. The ground leaves were stored in air-tight containers until needed.

Organisms used

S. aureus ATCC 29213, P. aeruginosa ATCC 27853, E. faecalis ATCC 29212 and Escherichia coli ATCC 25922 species are the major cause of nosocomial infections in hospitals (Sacho and Schoub, 1993) and are mainly the strains recommended for use by the National Committee for Clinical Laboratory Standards (NCCLS,

1992).

Extraction procedure

The ground leaves (1 g) were separately extracted with 10 ml of hexane, dichloromethane, acetone and methanol, using 50 ml centrifuge tubes. Extraction was performed three times per leaf sample. The extracts were filtered through Whatman no. 1 filter paper into universal bottles. The solvents were evaporated by placing the filtrates under a fan.

Phytochemical analysis

The plant extracts were dissolved in acetone to give a final concentration of 10 mg/ml. For each plant 10 μ l (100 μ g) was loaded on aluminium-backed thin layer chromatography (TLC) plate (Sigma) and the plate was developed in three solvent systems that is benzene/ethanol/ammonium solution (18:2:0.2) [BEA] (non polar/basic): chloroform/ethyl acetate/formic acid (10:8:2) [CEF] (intermediate polarity/acidic): ethyl acetate/methanol/water (10:1.35:1) [EMW] (polar/neutral) (Kotze and Eloff, 2002). The plates were viewed under ultra violet (UV) light (254 and 365 nm) for compounds which are fluorescent and later sprayed with vanillin (Sigma) sulphuric acid reagent and heated to visualize colors of the different compounds in each extract (Figure 2).

Antibacterial activity

Quantitative antibacterial activity assay by minimum inhibitory concentration (MIC)

The serial microplate dilution method developed by Eloff (1998a) was used to determine minimum inhibitory concentration (MIC) values. Four bacterial species were used for the assay, E. coli E. faecalis, P. aeruginosa and S. aureus. Each organism was inoculated into 150 ml of nutrient broth, incubated for 24 h at 37°C and was used as the stock culture. The organisms (10 ml) were further inoculated in 100 ml of nutrient broth and incubated at 37°C for 24 h. The plant residues were re-dissolved in acetone to give a final concentration of 10 mg/ml and 100 µl of each plant extract was serially diluted (50%) with water in a 96 well microtitre plate. Hundred microlitres of bacterial culture was added to each well (Table 2). The plates were incubated at 37°C for 24 h. Similar dilution of ampicillin (Sigma) was used as the positive control. After incubation, 40 µl of p-iodinitrotetrazolium violet (INT) (Sigma) dissolved in water was added to each of the microplate wells to detect growth. Bacterial growth inhibition was indicated by the reduction of the purple color. The total activity of the extracts in ml/g was calculated by dividing the MIC value with the quantity extracted from 1 g of plant material (Table 3). The resultant value indicates the volume to which the extract can be diluted and still inhibit the growth of a microorganisms (Eloff, 2004).

Qualitative antibacterial activity assay by bioautography

The bioautography procedure described by Begue and Kline (1972) was used to determine the number of antibacterial compounds. The TLC plates were loaded with 20 μ l (200 μ g) of each plant extract dissolved in acetone. The plates were developed in solvent systems as used in phytochemical analysis. After development the plates were placed under a stream of air for a period of 5 days to allow the solvents to evaporate. The plates were sprayed with the various cultures, separately, until the plates were just wet and then incubated at 37°C for 24 h. The plates were then sprayed with INT

Botanical name	Family	Application
Ricinus communis	Euphorbiaceae	Used to treat warts, cold tumours, indurations of the abdominal organs etc. (Carvalha et al., 1997).
Ziziphus mucronata	Rhamnaceae	Root and leave pastes applied on boils, swollen glands, wounds and sores (Mthethwa et al., 2009).
Lippia javanica	Verbenaceae	Used to treat skin rashes, stings and bites (Bussmann et al., 2006).
Lantana camara	Verbenaceae	Used to treat itches, cuts, ulcers, swelling, and eczema and wound healing (Raina et al., 2008).
Senna italic	Fabaceae	Leaves used as dressing for skin problems such as burns and ulcers (Masoko et al., 2010).

Table 1. Plants used in this study and their applications.

Table 2. MIC values of the selected plant extracts in mg/ml.

Organism	S. italica	L. javanica	R. communis	Z. mucronata	L. camara	<i>Ampicillin</i> (µg/ml)
E.coli	1.25	0.64	1.25	0.53	-	0.16
E. faecalis	1.25	0.64	0.84	1.05	-	0.16
P.aeruginosa	0.84	0.32	-	-	-	0.13
S. aureus	0.63	0.64	-	0.53	-	0.08
Average	0.99	0.56	1.05	0.70	-	0.13

- indicates no activity.

Table 3. Total activity of the selected plant extracts (ml). Total activity was calculated as the mass of the plant extract divided by the MIC value.

Organism	S. italica	L. javanica	R. communis	Z. mucronata	L. camara
E.coli	20	127	77	60	-
E. faecalis	20	127	114	30	-
P.aeruginosa	30	253	-	-	-
S. aureus	40	127	-	60	-
Average	28	159	96	50	-

- indicates no activity.

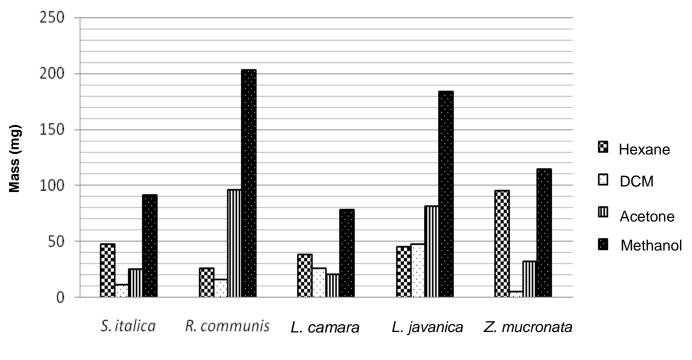
(concentration) and incubated for 30 min. The presence of clear bands on the plates against a purple background indicated growth inhibition (Begue and Kline, 1972).

Qualitative 2, 2-diphenyl-1-pacrylhydrazyl (DPPH) assay on TLC

TLC plates were used to separate extracts as described. The plates were dried in the fume-hood. To detect antioxidant activity, chromatograms were sprayed with 0.2% 2,2-diphenyl-2-picrylhydrazyl (DPPH) (Sigma) in methanol, as an indicator. The presence of antioxidant compounds was detected by yellow spots against a purple background on TLC plates sprayed with 0.2% DPPH in methanol.

RESULTS AND DISCUSSION

Majority of traditional healers use readily available water for extraction since it is readily available and it is nontoxic. In this study, the extraction process was carried out using different solvents. The problem with using water is that non-polar active compounds cannot be extracted (Masoko et al., 2008). All the extracts were placed under a fan to evaporate the solvents, after which the extracts were re-dissolved in acetone because acetone has been found to be a good solvent for both polar and non-polar compounds and it has been demonstrated that under similar test conditions not harmful to organisms such as



Selected plants

Figure 1. The mass of the plants extracted using different solvents (extraction process).

fungi (Masoko et al., 2007) and bacteria (Eloff, 1998b). Methanol was found to be the best extractant for all the plants since it extracted the greatest mass (Figure 1). In the phytochemical analysis, more bands were observed in BEA, followed by CEF and EMW (Figure 2) which shows that most compounds are non-polar.

L. javanica extract with an overall average MIC value of 0.56 mg/ml was the most active. R. communis had the highest MIC average of 1.05 mg/ml and was only active against two bacterial species, namely E. coli and E. faecalis. This contradicted the findings on R. communis by Luseba et al. (2007) which showed good MIC values against S. aureus, E. coli and P. aeruginosa. They attributed the good activity to the presence of compounds such as tannins, flavonoids and indole-3-acetic acid (Luseba et al., 2007). In this study, S. aureus (Grampositive) and E. coli (Gram-negative) were the most sensitive, and P. aeruginosa (Gram-negative) the most resistant. P. aeruginosa is also associated with nosocomial infections and is difficult to eradicate due to its resistance to most antimicrobial agents (Todar, 2007). L. camara was not active against any of the organisms studied. Previous studies have shown that Gram-positive bacteria are most susceptible to extracts (Vlietinck et al., 1995).

From the bioautograms (Figure 3), *S. italica* and *Z. mucronata* had a high number of active compounds in BEA. CEF either had a few bands or there was no

bacterial growth due to formic acid. Masoko et al. (2008) reported that formic acid inhibit the growth of certain microorganisms. Preliminary screening studies by McGaw et al. (2007) using Z. mucronata extracts showed high levels of antibacterial efficacy. The compound 2,3dihydroxyl-up-20-en-28-oic acid was isolated from the plant and showed excellent activity against S. aureus which supports claims about the efficacy of Z. mucronata leaf paste in treating bacterial infections in both humans and animals (McGaw and Eloff, 2008). A literature survey on the chemical constituents of the genus Senna revealed the presence of alkaloids, guinines and anthraquinones (Masoko et al., 2010), and these compounds are suspected to be responsible for the biological activity of the plants in this genus. Previous studies also showed that 2,5-dihydroxy-3-methoxy-7methylanthraquinone isolated from S. italica showed activity against several Gram positive and Gram negative bacteria (Masoko et al., 2010).

The R_f values (Tables 4 to 7) of the zones of inhibition on the bioautograms were calculated. The clear zones were located at different R_f values on bioautograms suggesting that more than one compound is involved in the antibacterial activity. The values in Tables 8, 9 and 10 show that some of the zones of inhibition appeared in more than one plant and even in more than one solvent system. The presence of many bands in BEA shown in Table 4, suggests that nonpolar compounds have more

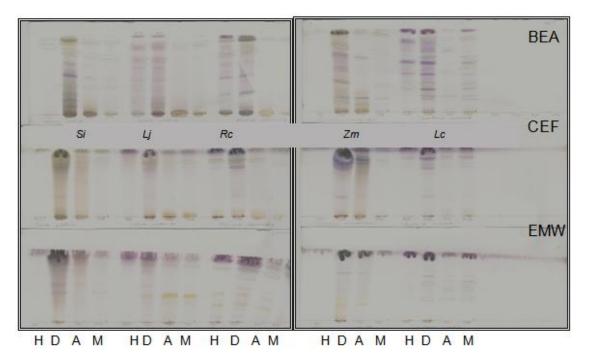


Figure 2. Chromatograms sprayed with vanillin sulphuric-acid reagent to show compounds extracted with hexane (H), dichloromethane (D), acetone (A) and methanol (M) from *Senna italica* (*Si*); *Lippia javanica* (*Lj*); *Ricinus communis* (*Rc*); *Ziziphus mucronata* (*Zm*) and *Lantana camara* (*Lc*) (phytochemical analysis).

Table 4. The inhibition of bacterial growth by bioautography of the extracts of <i>S.italica</i> , <i>L. javanica</i> and <i>R. communis</i> separated by TLC
with BEA as eluent. R _f values and relative degree of inhibition.

Divalue	E	BEA/ He	xane			BEA	/DCM		B	BEA/ A	Acetor	ne		BEA/ M	ethanc	bl
R _f value	E.c	E.f	S.a	P.a	E.c	E.f	S.a	P.a	E.c	E.f	S.a	P.a	E.c	E.f	S.a	P.a
						S	Senna ita	lica								
0.77								Х								
0.72							XXX	XXX				Х				
0.56					XXX	XXX	XXX	XXX								
0.46	XX				XXX				Х				XX	XXX		
0.31					XXX	XXX										
0.21					XXX		XXX	XXX								
	I					Li	ppia java	anica	I				I			
0.56							XXX									
0.46	XX				XX			XX								
0.31		Х	ХХ		XX	Х	XX	Х								
0.21		XXX				Х										
	I					Rici	nus con	nmunis	I				I			
0.77				Х			XXX	Х								
0.69							XX	Х								
0.51		XX		XX		XX	XX	XXX								
0.46					XX											
0.38					XXX											
0.31	XXX	Х			XXX	XXX		Х								
0.21		XXX	Х			XXX	Х	Х		Х						

E.c = *E. coli*, E.f = *E. faecalis*, S.a = S. *aureus*, P.a = *P. aeruginosa*. Relative degree of inhibition: X = slight inhibition, XX = moderate inhibition, XX = high inhibition. BEA = benzene:ethanol:ammonium solution.

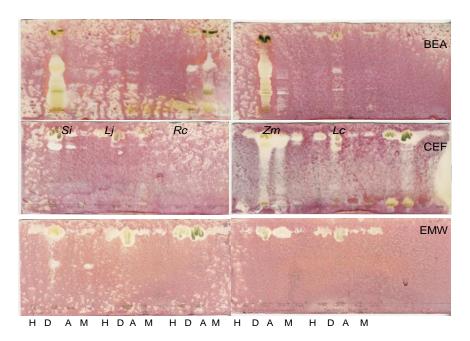


Figure 3. Bioautograms of selected plant extracted with hexane (H), dichloromethane (D), acetone (A), methanol (M), separated by BEA, CEF, and EMW and sprayed with *E. coli*. White areas indicate where reduction of INT to the coloured formazan did not take place due to the presence of compounds which inhibited the growth of *E. coli* (bioautography).

Table 5. The inhibition of bacterial growth by bioautography of the extracts of *Z. mucronata* and *L. camara* separated by TLC with BEA as eluent. R_f values and relative degree of inhibition.

		BEA/ H	lexane			BEA	DCM			BEA/	Acetone	9	B	EA/ Me	ethanc	bl
R _f values	E.c	E.f	S.a	P.a	E.c	E.f	S.a	P.a	E.c	E.f	S.a	P.a	E.c	E.f	S.a	P.a
						Zi	ziphus	mucror	nata							
0.81								XX								
0.7								XX								
0.57								XX								
0.48					XXX		XXX		Х		XXX					
0.42					XXX				XX							
0.38						XXX	XXX			XXX				Х		
0.35					XXX	XXX				XXX						
0.26					XXX	XXX	XXX	XXX		XXX		XXX				
0.19					XXX			XXX			XX	XXX				Х
0.16					XXX		XXX	XXX								
0.13					XXX	XXX		XXX			XX	XXX		Х		XX
							Lantan	a cama	ra							
0.97			Х				Х									
0.86							Х									
0.63		Х				XXX	Х	Х						Х		
0.51				Х		XX	Х	XX						Х		Х
0.48				Х	XXX			XX					х			ХХ
0.38				Х	Х			XX				Х	х			Х
0.26					Х									Х		
0.13		Х	Х			XXX	Х									

E.c = E. coli, E.f = E. faecalis, S.a = S. aureus, P.a = P. aeruginosa. Relative degree of inhibition: X =slight inhibition, XX = moderate inhibition, XXX = high inhibition. BEA = benzene:ethanol:ammonium solution.

Dualus		CEF/H	lexane	•		CEF/	DCM			CEF/Ac	etone			CEF/Me	thano	1
R_f value	E.c	E.f	S.a	P.a	E.c	E.f	S.a	P.a	E.c	E.f	S.a	P.a	E.c	E.f	S.a	P.a
							Sen	na italio	a							
0.73					XXX				XXX				Х			
0.62						XXX				XXX						
0.21						XXX				XXX				XXX		
	I						Lippi	ia javan	ica				I			
0.73					XX				х				ХХ			
0.69								XXX								
0.62						XX										
							Ricinu	s comm	unis							
0.72						XXX										
0.62						XXX										
	I						Ziziphu	s mucre	onata				I			
0.82						XXX				XXX				XX		
0.72							XXX				XXX					
0.67							XXX				XXX					
0.62							XXX				XXX				Х	
0.56					XXX											
1							Lanta	na cam	ara				l			
0.72							Х									
0.56					XX											

Table 6. The inhibition of bacterial growth by bioautography of the extracts of the selected plants separated by TLC with CEF as eluent. R_f values and relative degree of inhibition.

E.c = E. coli, E.f = E. faecalis, S.a = S. aureus, P.a = P. aeruginosa. Relative degree of inhibition: X =slight inhibition, XX = moderate inhibition, XXX = high inhibition. CEF = chloroform:ethylacetate:formic acid.

Table 7. The inhibition of bacterial growth by bioautography of the extracts of the selected plants separated by TLC with EMW as eluent. R_f values and relative degree of inhibition.

	E	EMW/H	lexan	e		EMW	//DCM		E	MW/A	Acetor	ne		EMW/Me	ethano	I
R _f values	E.c	E.f	S.a	P.a	E.c	E.f	S.a	P.a	E.c	E.f	S.a	P.a	E.c	E.f	S.a	P.a
							Senna	a italica	_							
0.71					XXX				Х				XXX			
0.63				XX		XXX		XXX		Х		Х		XXX		XXX
0.4							XXX									
							Lippia	javanica	1				I			
0.56						XX	XX	XX								
							Ricinus (commun	nis				l			
0.53						XX	XX	XX								
0.46		XX	XX	XX		XXX	XXX	XXX								
							Lantan	a camara	a							
0.44						Х	XX			Х				Х	XX	
0.31							XX							XX	XX	
0.18															ХХ	

E.c = E. coli, E.f = E. faecalis, S.a = S. aureus, P.a = P. aeruginosa. Relative degree of inhibition: X = slight inhibition, XX = moderate inhibition, XXX = high inhibition. EMW = ethyl acetate:methanol:water.

Durahua		BEA/	Hexan	е		BEA	VDCM		E	BEA/A	ceton	е	E	BEA/N	/lethar	nol	
R_f value	E.c	E.f	S.a	P.a	E.c	E.f	S.a	P.a	E.c	E.f	S.a	P.a	E.c	E.f	S.a	P.a	Total
0.97	-	-	-	1	-	-	1	-	-	-	-	-	-	-	-	-	2
0.86	-	-	-	-	-	-	1	-	-	-	-	1	-	-	-	-	2
0.81	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	1
0.77	-	-	-	1	-	-	1	2	-	-	-	-	-	-	-	-	4
0.72	-	-	-	-	-	-	1	1	-	-	-	1	-	-	-	-	3
0.7	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	1
0.69	-	-	-	-	-	-	1	1	-	-	-	-	-	-	-	-	2
0.63	-	1	-	-	-	1	1	1	-	-	-	-	-	-	1	-	5
0.57	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	1
0.56	-	-	-	-	1	1	2	1	-	-	-	-	-	-	-	-	5
0.51	-	1	-	2	-	2	2	2	-	-	-	-	-	-	1	1	11
0.48	-	-	-	-	2	-	1	1	1	-	1	-	-	-	-	1	7
0.46	2	-	-	-	3	-	-	1	1	-	-	-	1	1	-	-	9
0.38	-	-	-	1	2	-	-	1	-	-	1	-	1	1	-	1	8
0.35	-	-	-	-	1	1	-	-	-	1	-	1	-	-	-	-	4
0.31	1	2	1	-	3	3	-	2	1	-	-	-	-	-	-	-	13
0.26	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	1
0.21	-	2	1	-	1	2	2	2	-	1	-	-	-	-	-	-	11
0.19	-	-	-	-	1	-	-	1	-	-	1	1	-	-	-	-	4
0.16	-	-	-	-	1	-	1	1	-	-	-	-	-	-	-	-	3
0.13	-	1	1	-	-	-	-	-	-	-	1	1	-	-	-	-	4
Total	3	7	3	5	16	10	14	20	3	2	4	5	2	2	2	3	
Grand total			18				60			1	4			9			

Table 8. Number of antibacterial bands at different R_f values in all the five selected plants separated by BEA.

E.c = *E. coli*, E.f = *E. faecalis*, S.a = *S. aureus*, P.a = *P. aeruginosa*. Relative degree of inhibition: X = slight inhibition, XX = moderate inhibition, XXX = high inhibition. BEA = Benzene:ethanol:ammonium.

	(CEF/H	lexan	е		CEF	/DCM		C	EF/A	ceton	е	С	EF/M	ethan	ol	
R _f value	E.c	E.f	S.a	P.a	E.c	E.f	S.a	P.a	E.c	E.f	S.a	P.a	E.c	E.f	S.a	P.a	Total
0.82	-	-	-	-	-	1	-	-	-	1	-	-	-	1	-	-	3
0.73	-	-	-	-	2	-	-	-	2	-	-	-	2	-	-	-	6
0.72	-	-	-	-	-	1	1	-	-	-	1	-	-	-	-	-	3
0.69	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	1
0.67	-	-	-	-	-	-	1	-	-	-	1	-	-	-	-	-	2
0.62	-	-	-	-	-	3	1	-	-	1	1	-	-	-	1	-	7
0.56	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	1
0.21	-	-	-	-	-	1	-	-	-	1	-	-	-	1	-	-	
Total	0	0	0	0	3	6	3	1	2	3	3	0	2	2	1	0	3
Grand total		(0			1	3				В			!	5		

Table 9. Number of antibacterial bands at different Rf values in all five selected plants separated by CEF.

E.c = *E. coli*, E.f = *E. faecalis*, S.a = *S. aureus*, P.a = *P. aeruginosa*. Relative degree of inhibition: X = slight inhibition, XX = moderate inhibition, XXX = high inhibition. CEF = chloroform:ethylacetate:formic acid.

antibacterial activity. More zones of inhibitions were observed in DCM, suggesting that the solvent was able to extract many active compounds. The non-activity of the polar extracts in bioautography is thought to be attributed to the evaporation of volatile active compounds during removal of the TLC eluents or disruption of synergism between active constituents caused by TLC separation (Masoko and Eloff, 2006). The DPPH method measures

D volue	E	EMW/I	Hexan	е		EMW	/DCM		E	MW/A	Acetor	ne	El	MW/M	lethan	ol	Total
R _f value	E.c	E.f	S.a	P.a	E.c	E.f	S.a	P.a	E.c	E.f	S.a	P.a	E.c	E.f	S.a	P.a	Total
0.71	-	-	-	-	1	-	-	-	1	-	-	-	1	-	-	-	3
0.63	-	-	-	1	-	1	-	1	-	1	-	1	-	1	-	1	7
0.56	-	-	-	-	-	1	1	1	-	-	-	-	-	-	-	-	3
0.53	-	-	-	-	-	1	1	1	-	-	-	-	-	-	-	-	3
0.46	-	1	1	1	-	1	1	1	-	-	-	-	-	-	-	-	6
0.44	-	-	-	-	-	1	1	-	-	1	-	-	-	1	1	-	5
0.4	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	1
0.31	-	-	-	-	-	-	1	-	-	-	-	-	-	1	1	-	3
0.18	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	1
Total	0	1	1	2	1	5	6	4	1	2	0	1	1	3	3	1	
Grand total			4			1	6				4			ł	В		

Table 10. Number of antibacterial bands at different R_f values in all five selected plants separated by EMW.

E.c = E. coli, E.f = E. faecalis, S.a = S. aureus, P.a = P. aeruginosa. Relative degree of inhibition: X = slight inhibition, XX = moderate inhibition, XXX = high inhibition. EMW = ethyl acetate:methanol:water.

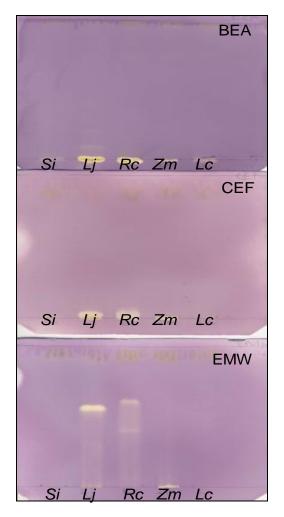


Figure 4. Chromatograms of the selected plants extracts separated by BEA, CEF and EMW solvent systems and sprayed with 0.2% DPPH. Si = *Sennaitalica*; Lj = *Lippia javanica*; Rc = *Ricinus communis*; Zm = *Ziziphus mucronata*; Lc = *Lantana camara* (antioxidant activity).

electron-donating activity of other compounds in the mixture and hence provides an evaluation of antioxidant activity due to free radical scavenging. Any molecule that can donate an electron or hydrogen to a mixture will react with and bleach DPPH. DPPH is reduced from a purple compound to a light yellow compound by electrons from oxidant compounds (Naik et al., 2003). The light yellow bands were observed on BEA and EMW plates. The active bands were present in *L. javanica* and *R. communis*, though not very active in BEA and in CEF no activity was observed (Figure 4) for all the plants.

Conclusion

The results indicate that the selected plants have antioxidant activity and antibacterial activity against the test organisms. This study serves as a scientific validation for the use of the plants in traditional medicine for wound healing. Further studies to isolate the antibacterial compounds are currently underway

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